

FIGURE 1

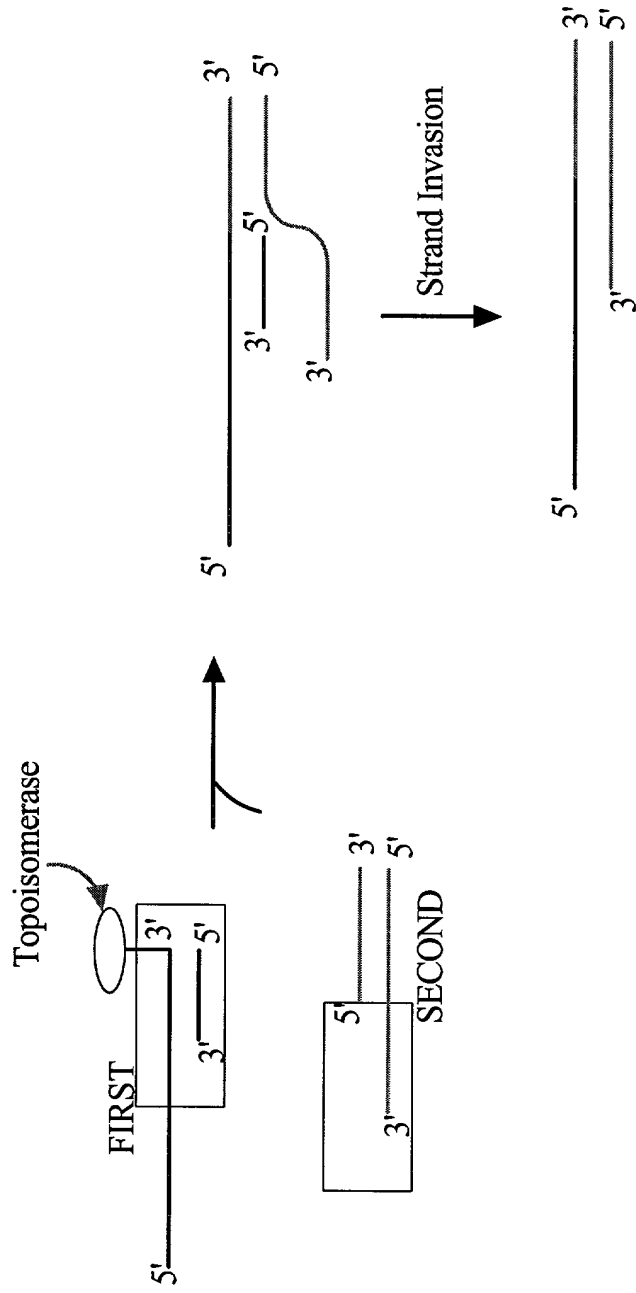


FIGURE 2

# MULTIPLE CLONING SITE



Uni1 Forward priming site

351 GAGCTTAGTA CGTACTATCA ACAGGTTGAA CTGCTGATCA ACAGATCCTC

Kpn I loxP site

401 TACGCGGCCG CGGTACC ATA ACT TCG TAT AGC ATA CAT TAT ACG

RBS Xho I RBS EcoR I Age I Bgl I

445 AAG TTA TCG GAGGAAT TGGCTCGAGG AATTCACCGG TGCCGTGTGG

BamH I Apa I Aat II Stu I Pvu I Sac I

491 GCGGATCCGG GCCCGACGTC AGGCC TCGAT CGGAG CTC GGT AAG CCT  
Gly Lys Pro

V5 epitope

538 ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT AGC CAT CAT  
Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Ser His His

6xHis tag Uni1 Reverse priming site

577 CAC CAT CAC CAT TGA AGCTCGCTA TCAGCCTCGA CTGTGCCTTC  
His His His His \*\*\*

621 TAGTTGCCAG CCATCTGTTG TTTGCCCCCTC CCCCCTGCCT

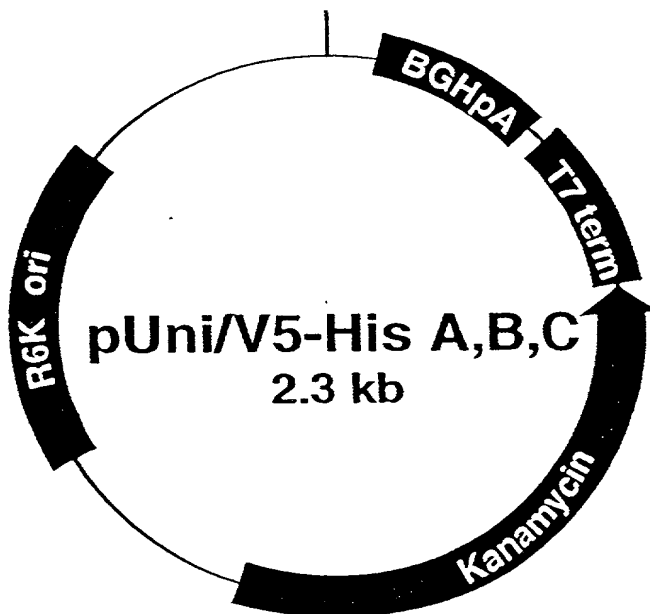


FIGURE 3

# **MULTIPLE CLONING SITE**

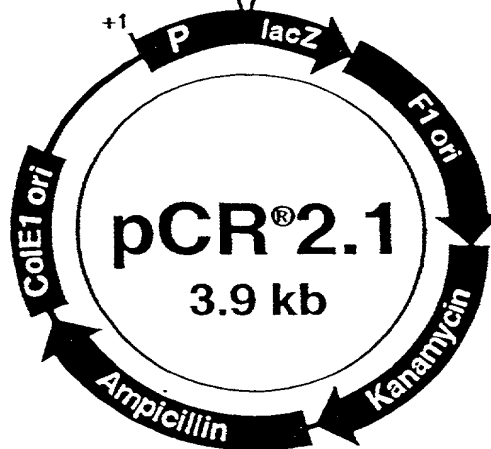
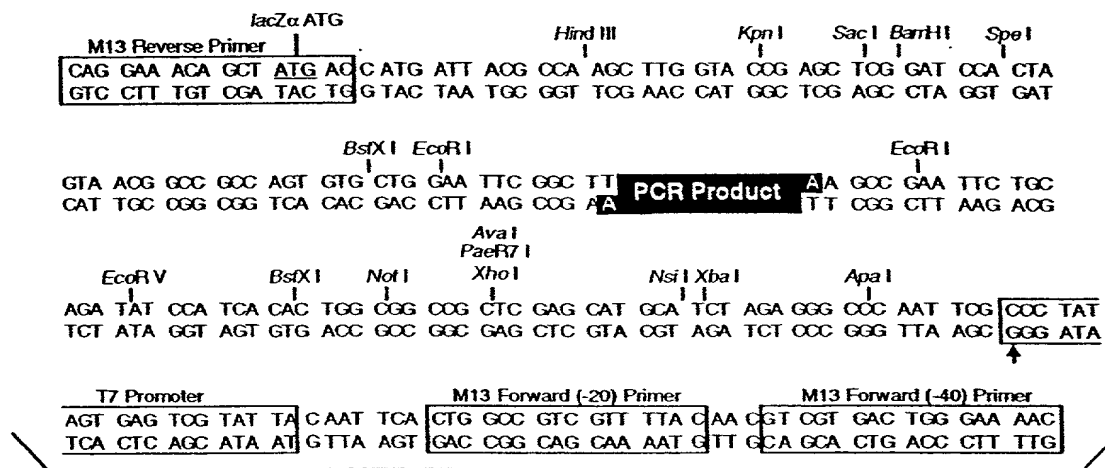
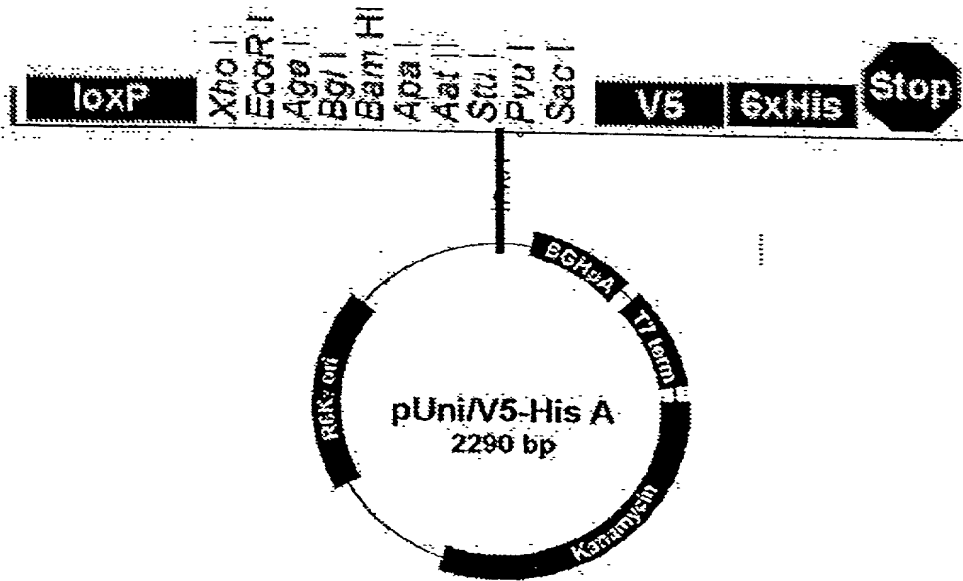


FIGURE 4

Sequence of pUni/V5-His version A

AATTC CATGTCAGCCGTTAAGTGTTCTGTGTC ACTCAAAATTGCTTTGAGAGGCTCTAAGGGCTTCTC  
 AGTGCGTTACATCCCTGGCTTGTTGCCACAACCGTTAAACCTTAAAAGCTTTAAAAGCCTTATATATTC  
 TTTTTTTCTTATAAACTTAAACCTTAGAGGCTATTTAAGTTGCTGATTTATATTAATTTTATTGTTT  
 AAACATGAGAGCTTAGTACGTGAAACATGAGAGCTTAGTACGTTAGCCATGAGAGCTTAGTACGTTAGCC  
 ATGAGGGTTTAGTTCTGTTAAACATGAGAGCTTAGTACGTTAAACATGAGAGCTTAGTACGTGAAACATGA  
 GAGCTTAGTACGTACTATCAACAGGTTGAACTGCTGATCAACAGATCCTCTACGCGGCCGCGGTACCATA  
 ACTTCGTATAGCATACATTATACGAAGTTATCGGAGGAATTGGCTCGAGGAATTCACCGGTGCCGTGTGG  
 GCGGATCCGGGCCCGACGTCAGGCCTCGATCGGAGCTCGGTAAGCCTATCCCTAACCCCTCTCCTCGGTCT  
 CGATTCTAGCCATCATCACCATCACCATTGAAGCTCGCTATCAGCCTCGACTGTGCCCTCTAGTTGCCAG  
 CCATCTGTTGTTTGCCCCCTCCCCCGTGCTTCCTTGACCCCTGGAAGGTGCCACTCCCACTGTCTTTCTCT  
 AATAAAATGAGGAAATTGCATCGCATTGTTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCA  
 GGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATTCTAGAAGATCCGGCTGCTA  
 ACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGG  
 CCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAAGTATATCCGGATATCCCGGGGTGGGCGA  
 AGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATCCAGCCGGCGTCCCGGAAAACGATTCCGAAGC  
 CCAACCTTTCATAGAAGGGCGCGGTGGAATCGAAATCTCGTGATGGCAGGTTGGGGCGTCGCTTGGTTCGGT  
 CATTTCGAACCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAA  
 TCGGGAGCGCGGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATA  
 TCAGGGGTAGCCAACGCTATGTCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAG  
 AAAAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGTGTACGACGAGATCCTCGCC  
 GTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTTCGGCTGGCGCGAGCCCTGATGCTCTTCGTCCAGA  
 TCATCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTC  
 GAATGGGCAGGTAGCCGATCAAGCGTATGCAGCCGCCGATTGCATCAGCCATGATGGATACTTTCTCG  
 GCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCC  
 GCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCCGTGCTGGCCAGCCACGATAGCCGCGCT  
 GCCTCGTCTGCAGTTCAATCAGGGCACCGGACAGGTTCGGTCTTGACAAAAAGAACCGGGCGCCCTGCG  
 CTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTTCTGTTGTGCCCAGTCATAGCCGAATAGCCT  
 CTCCACCAAGCGGCCGAGAACCTGCGTGCAATCCATCTTGTTCAATCATGCGAAACGATCCTCATCCT  
 GTCTCTTGATCAGATCTTGATCCCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTT  
 GCAGGGCTTCCCAACCTTACCAGAGGGCGCCCCAGCTGGCAATTCCGGTTTCGCTTGTGTCCATAAAACC  
 GCCAGTCTAGCTATCGCCATGTAAGCCCCACTGCAAGCTACCTGCTTTCTCTTTGCGCTTGGCTTTTCCCTT  
 GTCCAGATAGCCAGTAGCTGACATTCATCCGGGGTCAGCACCGTTTCTGCGGACTGGCTTTCTACGTGTT  
 CC GCTTCCTTAGCAGCCCTTGCGCCCTGAGTGCTTGGCGCAGCGTGAAGCT

FIGURE 5



Add EcoRI and SacI digestion enzymes

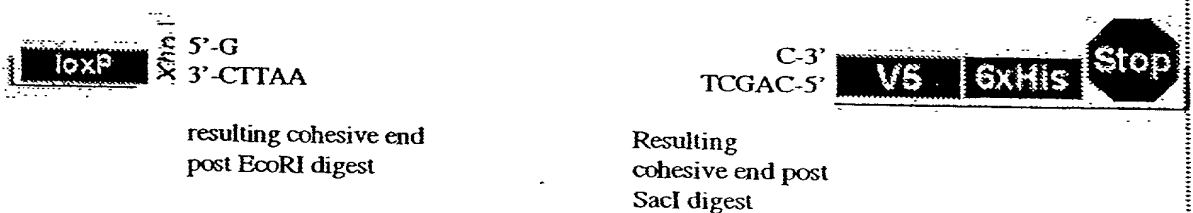


FIGURE 6

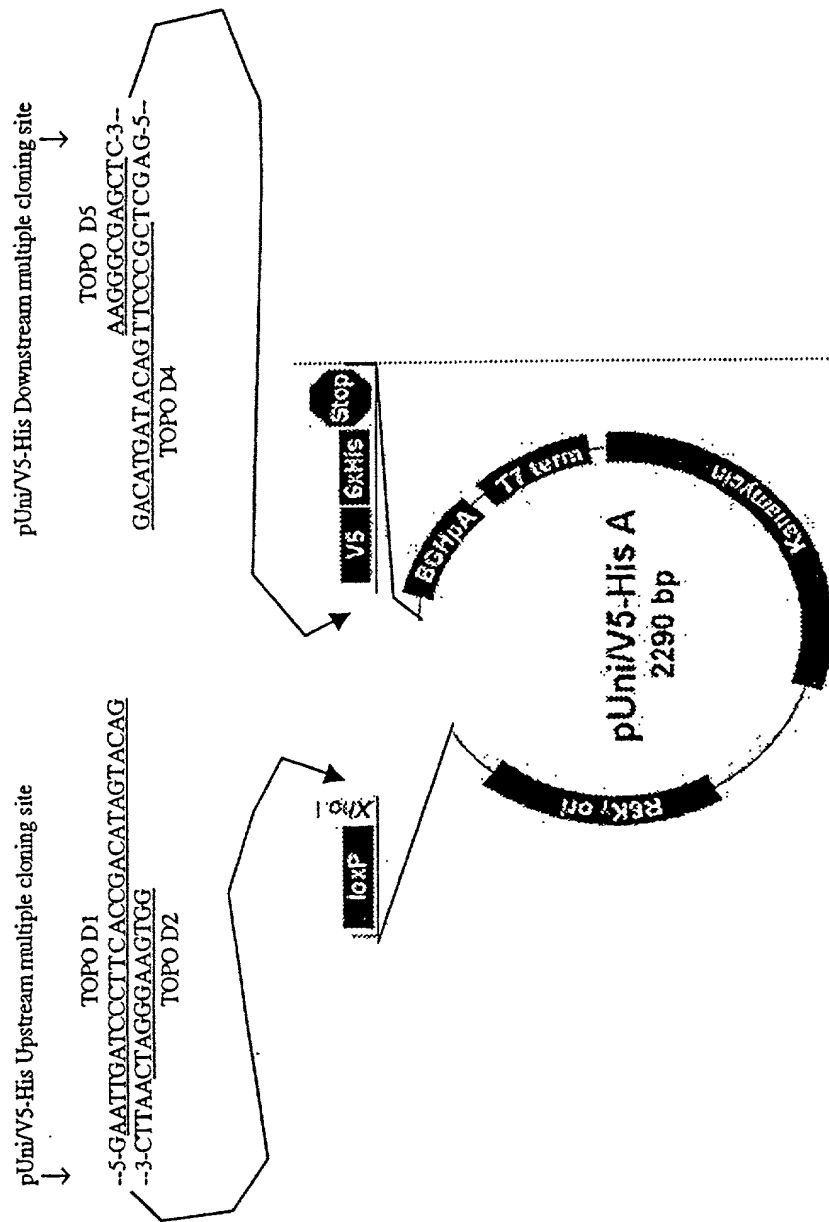


FIGURE 7

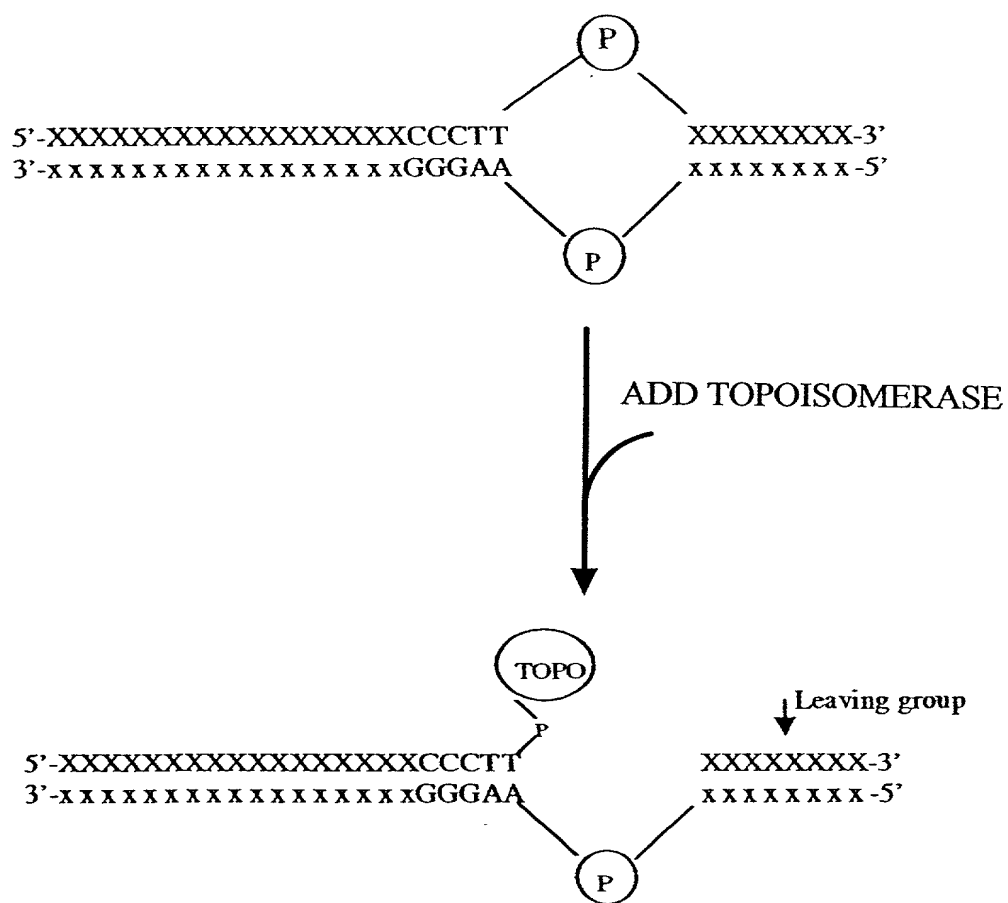


FIGURE 8



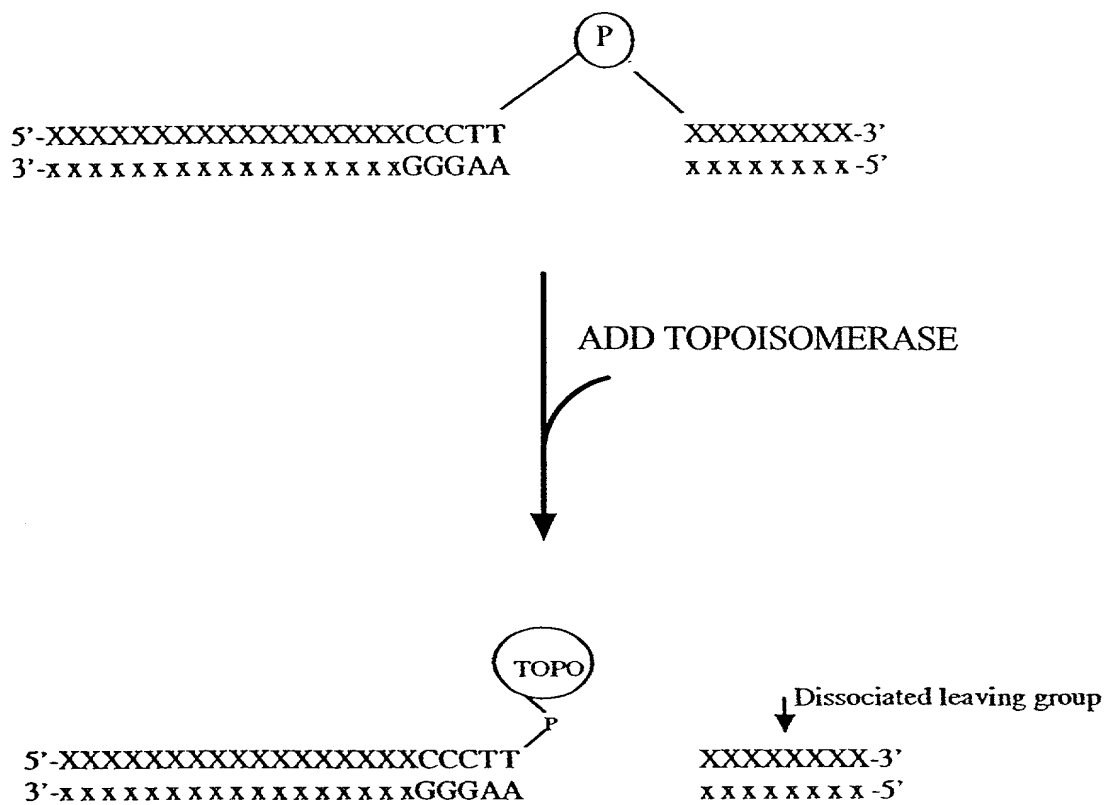


FIGURE 9

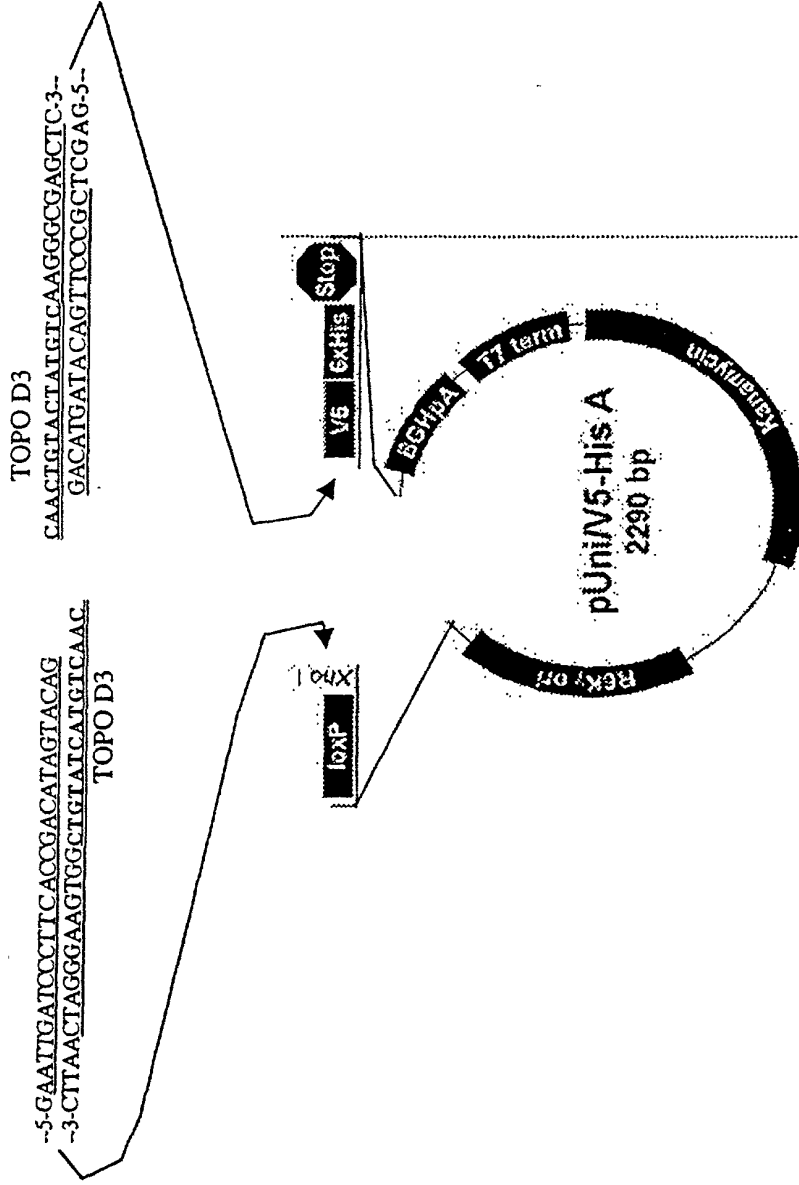


FIGURE 10

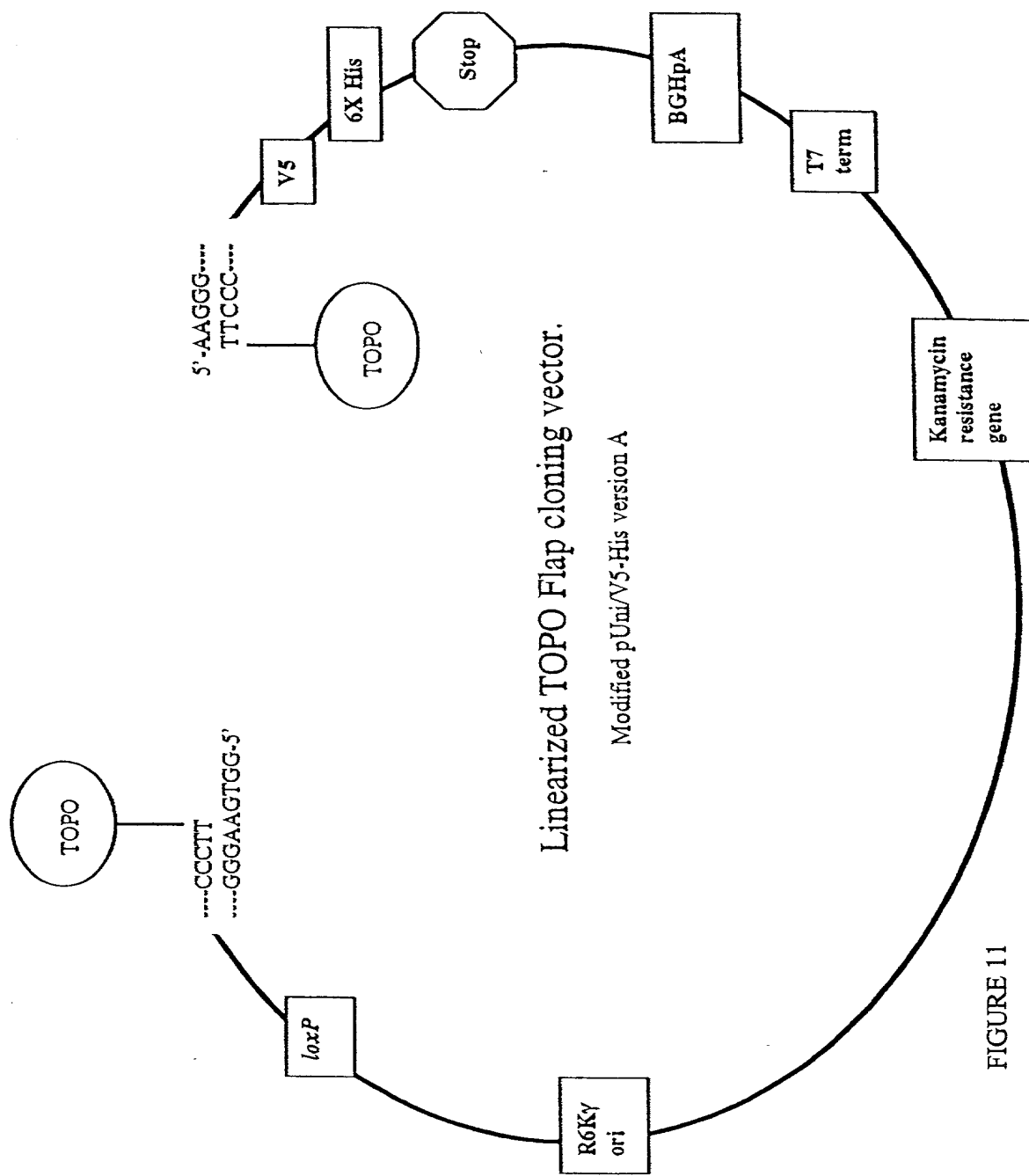


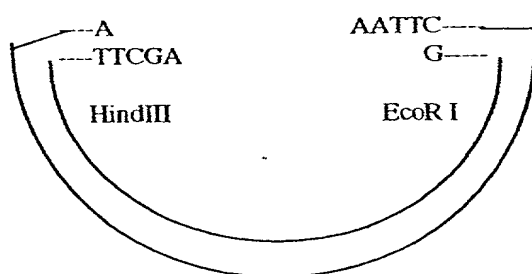
FIGURE 11

# Sequence of pCR 2.1

AGCGCCCAATACGCCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATATGCAGCTGGCAGCAGAGGTT  
TCCCGACTGGAAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAG  
GCTTTACACTTTTATGCTTCCGGCTCGTATGTTGTGTGGAAATTGTGAGCGGATAACAATTTACACAGGAA  
ACAGCTATGACCATGATTACGCCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGC  
TGGAATTCGGCTTAAGCCGAATTCCTGCAGATATCCATCACACTGGCGGGCGCTCGAGCATGCATCTAGAG  
GGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCAGTGGCCGTCGTTTACAACGTCGTGACTGGGA  
AAACCCCTGGCGTTACCCAACCTAATCGCCTTGCAGCACATCCCCCTTTCCGCCAGCTGGCGTAATAGCGAA  
GAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCGAGCCTGAATGGCGAATGGGACGCGCCCTGAGCG  
GCGCATTAAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGGCAGCGCCCTAGCGCC  
CGCTCCTTTCGCTTTCCTCCCTTCCTTTCCTGCCACGTTCCGCCGGCTTTCCCGTCAAGCTCTAAATCGG  
GGGCTCCCTTTAGGGTTCGGATTTAGAGCTTTACGGCACCTCGACCGCAAAAAAATTGATTTGGGTGATG  
GTTACAGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAA  
TAGTGGACTCTTGTTCCAAAGTAACTGGAACAACACTCAACCCCTATCGCGGTCTATTCTTTTGATTTATAAGGG  
ATTTTGGCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTAAACAAATTCAGGGCGAAAGGGCTGCTA  
AAGGAACCGGAACACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTGAGCTACTGG  
GCTATCTGGACAAGGGAACCGCAAGCGCAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGAT  
AGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCAGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGT  
TGGAAGCCCTGCAAAAGTAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGA  
TCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGG  
CCGCTTGGGTGGAGAGGCTATTCCGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGT  
GTTCCGGCTGTGACGCGAGGGGCGCCCGGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAA  
CTGCAGGACGAGGCGCGCGCTATCGTGGCTGGCCACGACGGGCGTTCCCTTGGCGAGCTGTGCTCGACG  
TTGTCAGTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCTATCTCG  
CCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCT  
ACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAAGGATGGAAGCCGGTCTTG  
TCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTGTCCGCCAGGCTCAAGGC  
GCGCATGCCCGACGGCGAGGATCTCGTCTGTATCCATGCGGATGCGCTGCTTGGCGAATATCATGGTGGA  
AATGGCCGCTTTTCTGGATTCAACGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATGCGGCT  
TGGATAACCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCTGCTGCTTTACGGTAT  
CGCCGCTTCCCGATTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTCTTCTGAATTGAAAAAGGA  
AGAGTATGAGTATTCACACTTTCGGTGTCCGCTTATTCCTTTTTCGCGCATTTTGCCTTCCTGTTTTT  
TGCTCACCCGAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATC  
GAAGTGGATCTCAACAGCGGTAAAGATCCTTGAGAGTTTTCGCCCGAAGAACGTTTTTCCAAATGATAGCA  
CTTTTAAAGTTCTGCTATGTCATACACTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCCCG  
GGCGCGGTATTCTCAGAATGACTTGGTTGAGTACTACAGATCACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAA  
CGATCGGAGGACCGAAGGAGTAAACCGCTTTTTCACACAACATGGGGATCATGTAACCTCGCCTTGATCG  
TTGGGAACCGGAGCTGAATGAAGCCATACCAACGACGAGAGTGACACCACGATGCTGTAGCAATGCCA  
ACAACGTTGCGCAAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGA  
TGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAA  
ATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAG  
GTGCCCTCACTGATTAAGCATTTGGTAAGTGTGACACCAAGTTTACTCATATATACTTTAGATTGATTTAAA  
ACTTCATTTTAAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAA  
CGTGAGTTTTCGTTCCACTGAGCGTCAGACCCGTAAGAAAGATCAAAAGGATCTTCTTGAGATCCTTTT  
TTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCA  
AGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAACTAGTCTCTTCTA  
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACCTGTAGCACCCGCTACATACTCTGCTGCTAATCC  
TGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTGTGCTTACCAGGGTTGGACTCAAGACGATAGTTACC  
GGATAAGGCGCAGCGGTCCGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTAC  
ACCGAAGTGAATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACA  
GGTATCCGGTAAGCGGACGGGTCCGAACAGGAGAGCGCAGAGGGAGCTTCCAGGGGGAACCGCTGGTA  
TCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGACGCTCGATTTTGTGATGCTGCTGCTGCTGCTC  
CGGAGCCTATGGAACAAACGCCAGCAACGCGGCCCTTTTACGGTTCCCTGGCCCTTTGCTGGCCCTTTGCTC  
ACATGTTCTTCTGCTGCTTATCCCTGATCTGTGATAACCGTATTACCGCCTTTGAGTGAGCTGATAC  
CGCTCGCCGACCGCAACGACCGAGCGCAGCGAGTCAAGTGAAGCGAAGCGGAAG

FIGURE 12

**A**



**B**

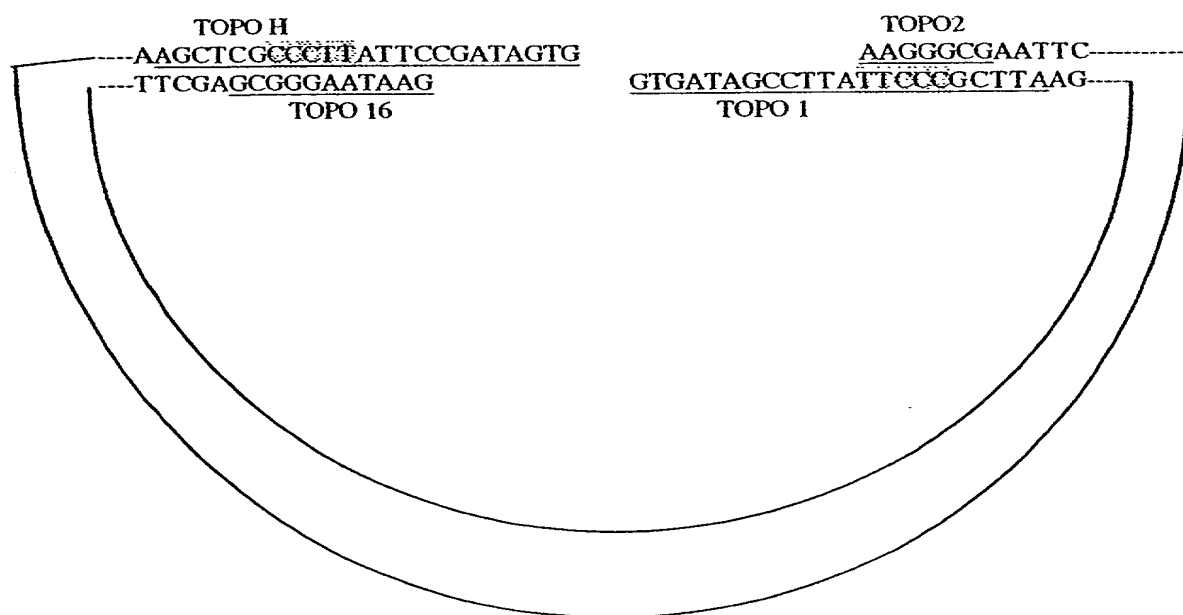


FIGURE 13



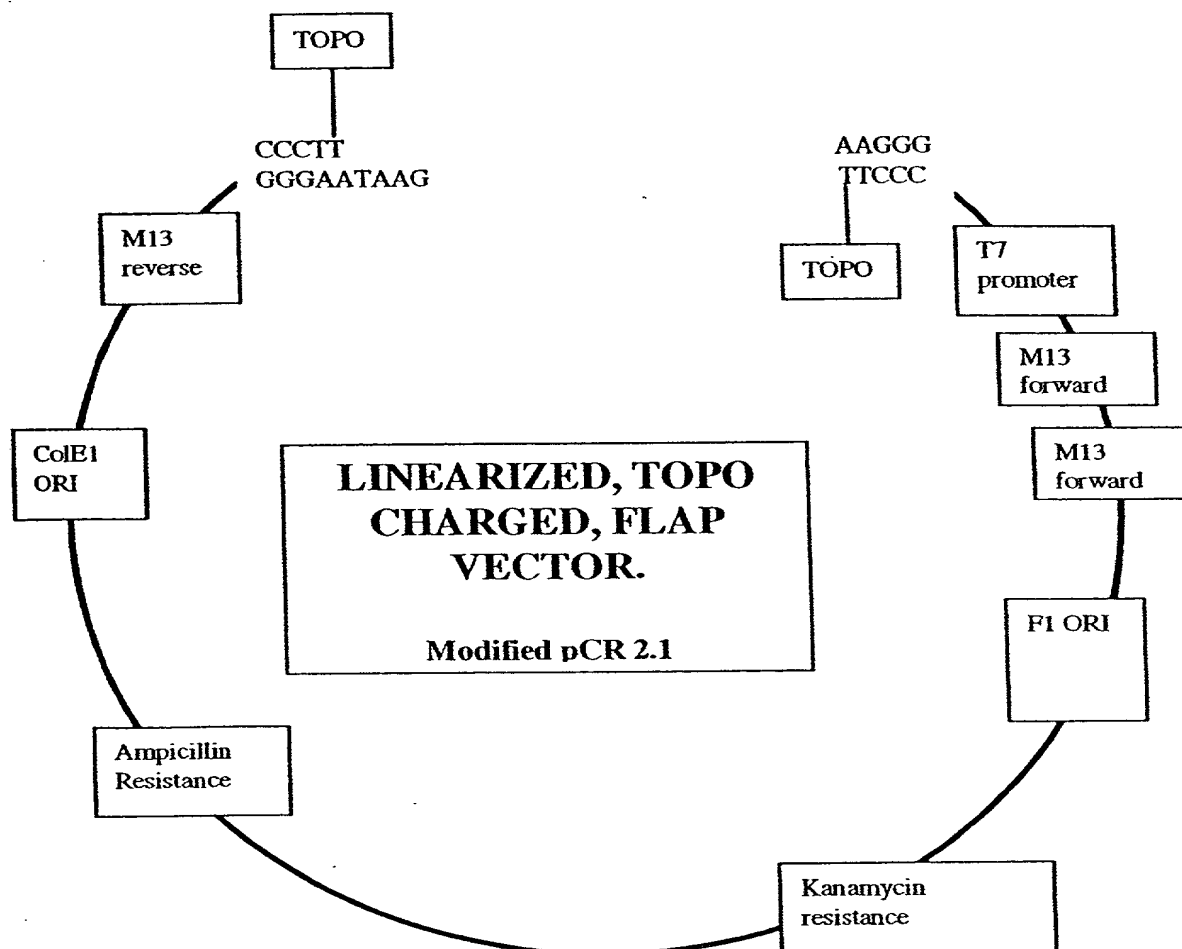


FIGURE 15





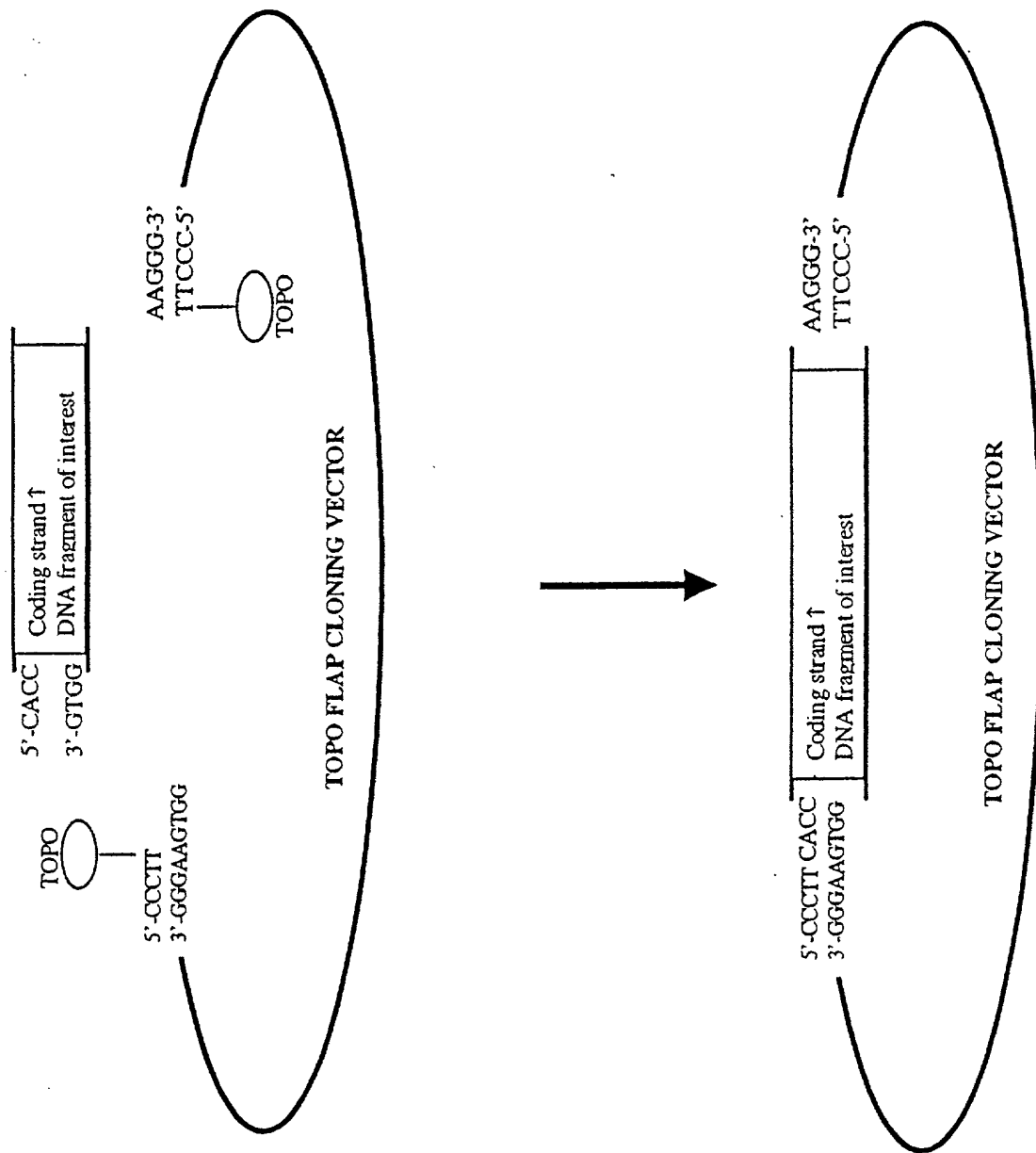


FIGURE 17